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Evaluation of a Continuous Liquid-Liquid Extractor for Isolation and Concentration of Nonpolar Organics for Biological Testing in the Presence of Humic Materials

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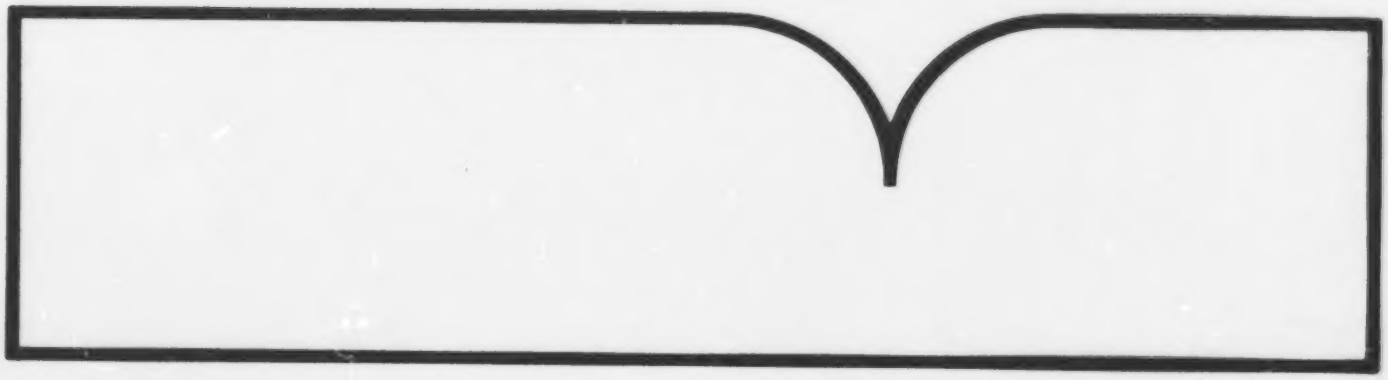

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16. ABSTRACT

A continuous liquid-liquid extraction system (CCLE) for concentrating trace organics from water into methylene chloride for analysis was designed, built and evaluated. The CLLE uses Teflon coils for phase contact and gravity phase separation. The system includes a self-contained excess solvent distillation chamber, so the methylene chloride is recovered and recycled.

Fourteen organic model compounds in the presence of humic material were used as test probes to evaluate the CLLE. Recovery values were determined for these compounds using CLLE and batch LLE (separatory funnel liquid-liquid extraction), and CLLE recoveries were found to be similar to those of batch LLE.

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Introduction

Biological testing undertaken to assess the health significance of potable water supplies usually requires the exposure of test species to chemicals at levels higher than those present in the water being tested. One example is the Ames test for mutagenic activity. Direct biological testing of drinking water is not now possible, as the total concentration of organic matter in water supplies is usually much less than 10 mg/L as dissolved organic carbon (DOC). It is estimated that 90% of the DOC in drinking water supplies is high molecular weight natural humic material which is apparently nontoxic (NAS, 1977). The remaining 10% of the DOC is characterized as low molecular weight organics. Many identifications have been made of the volatile organic fraction of the low molecular weight organics by gas chromatography/mass spectroscopy (GC/MS), whereas only 5-10% of the non-volatile organics comprising the remaining 90% of the DOC has been identified (NAS, 1980).

A problem often overlooked in potable water evaluation is that the complement of organic compounds, especially from a surface water source changes over time. The compounds present and their concentrations are constantly changing (Suffet et al., 1978). Thus, it is almost impossible to

formulate a "typical mixture" of organic compounds for a biological testing program. Therefore, a concentrated composite sample representing the water over a period of time should be used for any biological testing program of potable water. This is especially important for trace organic chemicals where the chronic effect is the primary concern. Thus, large volume composite samples taken over long periods of time are more representative of the chronic exposures presented to the public.

A recent National Academy of Science (NAS) review (NAS, 1983) of the available methods for preparation of water concentrates concluded that no single concentation method is adequate for isolating all the organic constituents from an aqueous sample. Each method suffers from differences in selectivity which are fundamental to that method. Artifacts added during processing are also a major concern. The EPA has recently evaluated several methods for preparing such concentrates from water for biological testing (Kopfler et al. 1981; Kopfler et al. 1985).

The isolation method of solvent extraction has been suggested as a potentially feasible process to concentrate trace organic compounds from finished drinking water (NAS 1983). One positive attribute of the solvent extraction method is that its performance for any given compound is theoretically predictable from a partition coefficient of a compound between the water sample and an organic solvent. The partition coefficient can be experimentally determined for any solute in any two phase solvent system (Suffet et al. 1972; Suffet et al. 1973). Variables of the extraction procedure such as solvent-to-water ratio and the choice of solvents can be adjusted to achieve optimum recovery.

Batch solvent extraction methods (e.g. by separatory funnel) for the preparation of extracts for biological analysis have a number of major

problems. A large volume of solvent must be evaporated after the extraction step in order to obtain a sample sufficiently concentrated to be useful for biological testing. Artifacts can occur from solvent impurities and reactions can occur during the evaporation process. Continuous liquid-liquid extractors (CLLEs) using a small amount of solvent which is continuously recycled would be more effective for this type of sampling. A CLLE system would be less effected by solvent contaminants and less solvent would be needed.

An on-line continuous liquid-liquid extraction system has been developed in our laboratories and has been used to isolate ng/l concentrations of organophosphate pesticides from natural waters (Wu and Suffet 1975, 1977) and to isolate a broad spectrum of gas chromatographable trace organics present in drinking water (Yohe et al. 1979, 1981, Suffet et al. 1980). An upgrade of the extractor has been recently developed (Baker et al. 1985). Features of the extractor include solvent recycle and adjustable water and solvent flow rates. The mixing of water and solvent is completed in a teflon coil. The solvent is separated from the aqueous phase by gravity and is recovered by distillation. All wetted parts are constructed of glass and teflon. The sampler can operate at 2 liters per hour as presently designed. A 100 liter sample concentrated to one mL would be needed for Ames testing, and would require 50 hours of extraction, followed by Kuderna-Danish evaporation to reduce the sample extract from 100 mL to one mL. This approach is now being studied to collect samples for the Department of Environmental Protection (DEP) of New Jersey at different water treatment plants in New Jersey (Suffet 1985).

Another scheme proposed for collecting enough concentrate for animal studies is shown in Figure 1. In this scheme for concentrating 135 gallons of water to a five gallon methylene chloride concentrate, reverse osmosis is

used to reduce the initial 2000 gallon volume of water to 135 gallons, and the CLLE is used to further concentrate the retentate to five gallons for biological testing. The LLE portion of this scheme was simulated in a set of CLLE evaluation experiments. The concentration factor from 135 to 5 gallons is 27:1, which was easily attainable with the CLLE units. Water to solvent ratio in the extractors is 10:1, and the additional volume reduction was achieved by solvent distillation in the distillation chambers of the CLLE units.

Experiments were designed to evaluate the performance of the CLLE units under conditions similar to those that may be encountered in field sampling while at the same time maintaining control over ionic strength, pH, salt matrix, trace organics present and humic content. Therefore, Milli-Q water spiked with salts, organic solutes and humics was used rather than natural water samples.

The objectives of this work were: (1) to evaluate the reliability and performance of this CLLE design in a large scale scheme for preparation of samples for biological testing, (2) to compare CLLE recovery to batch LLE recovery for a set of organic probes, (3) to evaluate variability in CLLE performance as a function of sample size, and above all (4) to evaluate the effects of background humic materials on the extraction process for both CLLE and batch LLE.

EXPERIMENTAL

CLLE Apparatus

Figure 2 is a schematic diagram of the extractor used in this work. Water and the extraction solvent (methylene chloride) are pumped together through a

T-joint, and the mixture flows through a mixing coil where extraction takes place. This coil consists of Teflon tubing (1/8 inch OD, 1/16 inch ID), 32 feet long wrapped around a one inch steel pipe for support.

The mixture exits the coil(s) and flows into the separation chamber, where the solvent and water mixture is separated by gravity. The lighter water phase rises, flowing through the upper outlet. The solvent phase is heavier and flows down through the main body of the extractor. A portion of it fills the float chamber, and the rest flows into the distillation chamber. Because they are hydraulically connected, the liquid level of the float and the distillation chamber remain equal while the extractor is in operation. A liquid level sensor (Therm-O-Watch Model L6-1000, I^2R Corp., Cheltenham, PA), clipped to the float chamber, electronically detects the presence or absence of a glass float which rises and falls with the methylene chloride level. When it rises high enough to be detected by the level sensor, a signal is sent to a Therm-O-Watch switching device which turns on the heater in the distillation chamber. When the solvent is distilled down to a preset minimum level the float falls below the level sensor and the heater is switched off. This on-off cycling continues during the entire extraction run, and the volume in the evaporation chamber remains relatively constant (70 to 80 ml). When the extraction run is completed, contents of the distilation chamber constitute the final extract. Initial versions of the extractor were designed by Wu and Suffet (1975; 1977) and Yohe and Suffet (1979). The most recent modification of the extractor has been described in detail by Baker et al. (1985).

Materials and Reagents

The materials and reagents for this work are described elsewhere in detail (Baker et al. 1985). The 14 nonpolar organic compounds and salt

solutions used in the CLLE evaluation are listed in Table 1.

Sample Concentration Experiments

A CLLE quality assurance blank was run by extracting 90 liters of Milli-Q water with three CLLE samplers in a parallel configuration and concentrating the composited extract to 4 ml by Kuderna-Danish evaporation. The 22,500 fold concentrate was analyzed by GC/MS, and only four contaminants were identified: cyclohexene, 2-cyclohexene-1-one, dibutyl phthalate and dioctyl phthalate. Cyclohexene is a solvent preservative that has been identified in commercial high purity methylene chloride (Keith et al. 1979), and 2-cyclohexene-1-one is its air oxidation product. The phthalates are ubiquitous laboratory contaminants and have also been identified in commercial methylene chloride (Bowers et al. 1981).

Three large scale water samples were prepared in 55 gallon stainless steel drums and used for evaluation of the CLLE units. Each solution was greater than a 100 liters and consisted of an aqueous mixture of inorganic salts and low molecular weight organic solutes. In the first experiment, the sample was adjusted to pH 7 whereas in the two other experiments the water was adjusted to pH 3. During the first experiment two batch and two continuous LLE were completed on 1.35 and 2.3 liters, respectively. The remaining 178 liters of sample was extracted by the three CLLE samplers used in a parallel configuration. The concentrates were continuously drained from each extractor through a needle valve and collected as a composite sample. The needle valves were set so that the final methylene chloride extract volume was 1/27 of the volume of aqueous solution extracted. This procedure served to assess the reliability of the units and the feasibility of using them in large scale concentration schemes as described in Figure 1. Sampling schemes for the second and third extraction experiment are shown in Figures 3

TABLE 1

COMPOUNDS USED FOR CLLE EVALUATION OF THE EFFECT OF HUMIC MATERIALS

COMPOUND	FUNCTIONAL GROUPS	COMPOUND	FUNCTIONAL GROUPS
ACETOPHENONE	KETONE	ISOPHORONE	KETONE
2,4-DICHLOROPHENOL	PHENOL	QUINOLINE	AMINE
BIPHENYL	ALKANE	ETHYL CINNAMATE	ESTER
DIACETONE-L-SORBOSE	SUGAR	1-CHLORODECANE	CL ALKANE
2-METHYL NAPHTHALENE	PAH	2,4-DICHLOROBIPHENYL	PCB
CAFFEINE	XANTHENE	ANTHRACENE	PAH
DI(ETHYL HEXYL) PHTHALATE	PHTHALATE	2,2',5,5'-TETRA-CHLOROBIPHENYL	PCB

COMPOUND	GROUPS	CONCENTRATION pH = 7	CONCENTRATION pH = 3
HUMIC ACIDS (FLUKA)	HUMICS	50 mg/l	50 mg/l
CALCIUM SULFATE	SALT	376 mg/l	1800 mg/l
SODIUM BICARBONATE	SALT	214 mg/l	1050 mg/l
CALCIUM CHLORIDE DIHYDRATE	SALT	144 mg/l	705 mg/l
IONIC STRENGTH		0.012	0.734

and 4. Several 12.5 liter CLLE extractions were run in the third experiment as shown in Figure 4. This allowed comparisons to be made of large versus small sample CLLE recoveries.

Humic materials (50 mg/L Fluka Humic Acids, Fluka Chemical Corp., Hauppauge, N.Y.) were added to the drum contents before the second half of each pH 3 extraction, Experiments 2 and 3, Figures 3 and 4. This allowed comparisons to be made between extractions with and without humics present.

Results and Discussion

First Experiment - pH = 7

The extractors performed without major interruptions or problems during the 178 liter, pH 7 extraction. The minor problems encountered were:

1. During the extraction, which was conducted over several days, significant growth of filamentous algae and bacteria occurred in the sample. The biomass accumulated in the phase separator, causing emulsification of the two phases. This resulted in some loss of sample concentrate, which would decrease recovery values for the trace organics. Lowering the pH to 3 during the subsequent two extraction experiments decreased but did not entirely eliminate the problem.

2. The glass floats, which control the liquid levels in the distillation chambers leaked and sank, causing problems with liquid level control in the distillation chambers. New floats were made, but minor leakage persisted.

3. Flow rate control of the solvent pump was difficult, since 0.2 liters per hour is near the low end of the solvent pump's capacity. This was resolved before the second experiment by changing the reduction gear ratio of the pumps.

4. Air bubbles in the system carried small amounts of solvent extract out the phase separator with the aqueous phase. This was eliminated by moving the pumps to a position lower than the other parts of the extractor, as recommended by the manufacturer, and ensuring that all teflon connections were air tight.

These problems were overcome by the end of the third extraction experiment, and the extractors can now be operated smoothly over long periods of time with no interuptions.

The recovery data for the CLLE and batch extracted portions at pH=7 are shown in Table 2. The limited number of data points (two each for batch and continuous extraction) made statistical analysis of the data impossible. However, it is clear that batch and continuous recoveries for most compounds are similar. The pH 7 solution used in the 45 gallon CLLE had biological growth in the drum which caused emulsification problems in the phase separator. Baker et al. (1985) showed that partition coefficients for nearly

TABLE 2

COMPARISON OF BATCH AND CONTINUOUS LLE PERCENT RECOVERY AT pH 7[a]

COMPOUND	BATCH[b] ± RANGE(%)	CLLE[c] ± RANGE(%)	178 L CLLE[d](%)
ACETOPHENONE	72 ± 5.0	82 ± 10.2	56
ISOPHORONE	78 ± 6.8	88 ± 11.1	81
2,4-DICHLOROPHENOL	52 ± 5.9	55 ± 3.6	61
QUINOLINE	69 ± 3.2	84 ± 5.6	87
BIPHENYL	60 ± 27.6	46 ± 7.0	28
CAFFEINE	40 ± 3.6	42 ± 4.3	28
DI(ETHYLHEXYL)PHTHALATE	101 ± 8.8	39 ± ---	35
**********	**********	**********	**********
2,2',5,5'-TETRACHLORO-BIPHENYL	114 ± 9.6	70 ± 14.0	134
2,4 DICHLOROBIPHENYL	41 ± 3.7	41 ± 7.6	30

******* UG/L RECOVERED FROM AQUEOUS SOLUTION (INITIAL CONC. UNK.)

a RANGE OF 2 VALUES

	NUMBER	SAMPLE VOLUME	WATER:SOLVENT RATIO	CONCENTRATION FACTOR
b BATCH LLE	2	1.35 L	10:1	10:1
c CLLE	2	2.32 L	10:1	10:1
d CLLE	1	178 L	10:1	27:1

NO HUMIC MATERIAL PRESENT

all of the test compounds are the same at pH 3 and 7, so extraction efficiency was not expected to decrease by lowering the pH in subsequent experiments. Therefore, pH 3 was chosen for the second and third experiment to minimize the effects of biological growth in the sample. This would not be a problem when continuous, large volume sampling is done directly from the water source into the extractors.

Second Experiment, pH = 3, Figure 3

The first pH 3 extraction was run continuously without major interuptions or operational problems. The phase separator had to be cleaned to remove algae and humic deposition four to five times during the 100 liter extraction. This problem would not occur during field sampling, because an on-line filter would be installed at the sample intake. Recovery values for batch and continuous extraction of this sample are listed in Table 3. The table includes recoveries before the addition of humics (previously reported in Baker, et al. 1985) and recoveries in the presence of humics.

A linear regression procedure (Figure 5) showed that one liter batch LLE is equivalent to one liter CLLE with and without humics present. Some compounds showed lower recoveries for 38 liter CLLE than for the one liter CLLE, but statistical evaluation of this was not possible since only one 38 liter extraction was run for each condition (with and without humics). The third experiment was designed to address the question of sample recovery, with and without the presence of humic materials.

Third Experiment, pH = 3, Figure 4

In this experiment it was determined that recoveries for one liter batch LLE, one liter CLLE and 12.5 liter CLLE samples are equivalent for 10 of 13 compounds tested without humics present (Baker, et al. 1985), and recovery

TABLE 3

PERCENT RECOVERY FOR CLLE AND BATCH EXTRACTION AT pH 3 WITH AND WITHOUT HUMIC MATERIAL- FIRST RUN

COMPOUND	1L W/O HUMICS BATCH [a] ± S.D.	1 L W HUMICS BATCH [b]	1 L W/O HUMICS CLLE [a] ± S.D.	1 L W HUMICS CLLE [c] ± S.D.	38 L W/O HUMICS CLLE [b]	38 L W HUMICS CLLE [b]
ACETOPHENONE	89 ± 4	79	85 ± 9	78 ± 4	86	79
ISOPHORONE	89 ± 4	88	86 ± 7	81 ± 6	97	98
2,4-DICHLOROPHENOL	73 ± 8	82	70 ± 13	66 ± 17	98	--
1-CHLORODECANE	25 ± 3	35	22 ± 4	24 ± 7	5	9
BIPHENYL	52 ± 2	23	45 ± 4	32 ± 2	39	22
CAFFEINE	37 ± 5	50	33 ± 10	41 ± 13	41	19
2,4 DICHLOROBIPHENYL	1090 ± 180	--	1000 ± 100	----	806	1042
2,2',5,5'-TETRACHLOROBIPHENYL	114 ± 32	179	99 ± 12	80 ± 20	63	82

******* UG/L RECOVERED FROM AQUEOUS SOLUTION (INITIAL CONC. UNK.)

a
Number of runs equal 6

b
Number of runs equal 1

c
Number of runs 3

differences for the other three are not substantial. Linear regression and ANOV procedures were used to make these comparisons.

The same statistical procedures were used here to evaluate the effects of humics on batch and continuous LLE. A base extraction procedure (Gibs, et al. 1985) was required for processing methylene chloride extracts prior to GC injection in order to protect the GC column from contamination by humics. This led to losses of 2,4-dichlorophenol and the chlorinated biphenyls. Therefore, these compounds were not used in the evaluation of the CLLE in the presence of humics. All other compounds were uneffected by the base extraction procedure. The ANOV procedure tested each compound for changes in concentration by comparing early sample recoveries to later ones. This was done separately for Parts #1 and #2. It was therefore possible to test each compound for decreasing concentration with and without the presence of humics.

Results of the nested ANOV for Part #1 (previously reported in Baker, et al. 1985) and Part #2 are listed in Table 4. The analysis shows that many of the compounds decrease in recovery over time. The following comparisons will be made for Experiment #3 data:

1. One liter batch LLE vs. one liter CLLE recoveries
   a. without humics
   b. with humics
2. Decrease in concentration of each compound over time in the pH 3 salt solution
   a. without humics
   b. with humics
3. Extraction with humics versus extraction without humics
   a. for batch LLE
   b. for CLLE

1. Batch LLE vs. CLLE

The linear regression procedure described earlier was also applied to the third experiment, and good linear relationships between batch LLE and CLLE recoveries for both Parts #1 and #2 of the experiment a) without humics and

TABLE 4

PERCENT RECOVERY FOR CLLE AND BATCH EXTRACTION IN THE PRESENCE OF HUMIC MATERIAL AT pH 3- EXPERIMENT #3

COMPOUND	1 L W/O HUMICS BATCH ± S.D. [a]	1 L W HUMICS BATCH [b]	1 L W/O HUMICS CLLE ± S.D. [c]	1 L W HUMICS CLLE ± S.D. [d]	12.5 L W/O HUMICS CLLE ± S.D. [e]	12.5 L W HUMICS CLLE ± RANGE [f]
ACETOPHENONE	94 ± 4	89 ± 6	101 ± 4	94 ± 9	89 ± 3	87 ± 12
ISOPHORONE	81 ± 8	79 ± 5	87 ± 6	83 ± 7	78 ± 3	88 ± 12
2,4-DICHLOROPHENOL	89 ± 10	----	94 ± 13	---	74 ± 8	---
QUINOLINE	27 ± 4	29 ± 3	27 ± 2	32 ± 3	25 ± 2	25 ± 5
1-CHLORODECANE	41 ± 3	38 ± 10	38 ± 9	36 ± 11	33 ± 6	19 ± 3
2-METHYLNAPHTHALENE	46 ± 9	23 ± 7	51 ± 8	27 ± 4	46 ± 3	32 ± 7
BIPHENYL	64 ± 4	52 ± 9	70 ± 2	54 ± 6	71 ± 3	50 ± 17
ETHYL CINNAMATE	88 ± 6	82 ± 8	96 ± 3	88 ± 8	90 ± 2	82 ± 14
DIACETONE-L-SORBOSE	50 ± 6	28 ± 9	55 ± 8	30 ± 7	46 ± 5	40 ± 24
ANTHRACENE	91 ± 34	61 ± 16	61 ± 3	65 ± 15	73 ± 16	47 ± 3
2,2',5,5'-TETRACHLORO-BIPHENYL	37 ± 9	------	22 ± 5	----	15 ± 3	---
2,4 DICHLOROBIPHENYL	174 ± 11	------	169 ± 15	----	148 ± 11	---

[a] SIX 1 LITER BATCH EXTRACTIONS

[b] SEVEN 1 LITER BATCH EXTRACTIONS

[c] EIGHT 1 LITER CLLES EXCEPT (7) FOR ANTHRACENE

[d] SEVEN 1 LITER CLLES

[e] FOUR 12.5 LITER CLLES EXCEPT (3) FOR ANTHRACENE

[f] TWO 12.5 LITER CLLES

******* UG/L RECOVERED FROM AQUEOUS SOLUTION (INITIAL CONC. UNK.)

b) with humics were shown (Figure 6). The ANOV procedure for Part #1 data (previously reported, Baker, et al. 1985) showed that mean recoveries for 10 of 13 compounds are homogeneous when one liter batch LLE, one liter CLLE and 12.5 liter CLLE comparisons are made. Slight recovery differences in the other three appear to be caused by minor losses in the CLLE distillation chamber during larger sample extractions, due to longer distillation time. One liter batch LLE and one liter CLLE samples were compared in Part #2, and eight of the nine compounds were found to have homogeneous mean recoveries (see Figure 6 Part #2). The one exception, quinoline, has low recovery values for both CLLE and batch LLE. This made the 3% difference between them (32 and 29%, respectively) proportionally large. This, combined with a small analytical error (also 3%) for quinoline resulted in the difference between means being considered significant by the ANOV procedure. Although this difference is statistically significant, it is not substantial enough to cause problems. Therefore, it has been shown that CLLE and batch recoveries are equivalent, with and without the addition of humics. When volume reduction is required after extraction (for CLLE or batch LLE), some evaporative losses will be experienced for some compounds.

2. Decreases in Concentration Over Time

The third experiment was run in two parts: during Part #1 no humic materials were present. Humics were added at the beginning of Part #2. The ANOV procedure tested each compound for changes in concentration by comparing early sample recoveries to later ones. This was done separately for Parts #1 and #2. It was therefore possible to test each compound for decreasing concentration with and without the presence of humics.

Results of the nested ANOV for Part #1 (previously reported in Baker, et al. 1985) and Part #2 are listed in Table 5. The analysis shows that many of

TABLE 5

ANOV COMPARISONS OF BATCH LLE and 1 LITER CLLE WITH AND WITHOUT HUMICS PRESENT, EXPERMENT #3

Compound	COMPOUND STABILITY ********** Ho #1 Non-humic	Ho #1 Humic	CLLE vs. BATCH LLE ********** Ho #2 Non-humic	Ho #2 Humic	HUMIC vs. NON-HUMIC ********** Ho #3 Batch LLE	Ho #3 CLLE
Acetophenone	Accept	Reject*	Reject*	Accept	Accept	Accept
Isophorone	Accept	Reject*	Accept	Accept	Accept	Accept
2,4-Dichlorophenol	Accept	(1)	Accept	(1)	(1)	(1)
Quinoline	Accept	Accept	Accept	Reject*	Accept	Accept
1-Chlorodecane	Accept	Accept	Accept	Accept	Accept	Accept
2-Methylnaphthalene	Reject*	Reject*	Accept	Accept	Reject*	Reject*
Biphenyl	Accept	Reject*	Accept	Accept	Accept	Reject*
Ethyl Cinnamate	Accept	Accept	Reject*	Accept	Accept	Accept
Diacetone-L-Sorbose	Reject*	Reject*	Accept	Accept	Reject*	Reject*
2,4-Dichlorobiphenyl	Accept	(1)	Accept	(1)	(1)	(1)
Caffeine (2)	Accept	(3)	Accept	(4)	(3)	(3)
Anthracene	Reject*	Accept	Accept	Accept	Accept	Accept
2,2',5,5'-Tetrachloro-biphenyl	Reject*	(1)	Accept	(1)	(1)	(1)

Hypotheses

Ho #1: Recovery of the compound does not decline over time (ANOV procedure)
Ho #2: Mean values of recovery for 1 liter batch LLE = 1 CLLE (ANOV procedure)
Ho #3: Mean value of recovery without humics present = mean value of reovery with humics present (ANOV procedure)

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(1) The chlorinated phenol and chlorinated biphenyls were not measured in the Humic portion of the experiment due to losses from the base extraction procedure.
(2) Caffeine data is from Experiment #2
(3) Insufficient data for ANOV procedure

the compounds decrease in recovery over time. Four compounds decreased during Part #1 (without humics) and three others declined after humics were added. Anthracene stopped declining after humics were added, which suggests a humic-anthracene association which protects anthracene from further losses. Only three of the compounds showed no decline in concentration over the entire duration of the experiment: quinoline, 1-chlorodecane and ethylcinnamate. These time-dependent recovery decreases would not occur during on-line sampling of a natural or treated water source, as any humic-organic interactions that result in removal of organics from aqueous solution would already have occurred, and humic-organic complexing would be in a state of equilibrium.

3. Extraction With Humics vs. Extraction Without Humics

Since the two parts of the experiment were run sequentially and not parallel, any decreases in concentration of test compounds over time would lead to lower apparent recoveries in Part #2. This had to be taken into account when comparisons were made between Part #1 and Part #2 (humic vs. non-humic) recoveries. Therefore, only recovery data for extractions imediately before and imediately after the addition of humics was used for this comparison (Ho #3 in Table 5). Figure 7 shows linear regressions of humic vs. non-humic recovery data for a) batch LLE and b) CLLE. Three different effects were observed for different compounds when humics were added to the sample:

1. Decreases in recoveries over time began after the addition of humics (3 compounds)
2. Decreases in recoveries over time ceased after the addition of humics (1 compound)
3. Recoveries were different for Part #1 (non-humic) and Part #2 (humic) for batch LLE, CLLE, or both (3 compounds)

Distribution of compounds within these three categories is shown in

Table 5. It is not known by what mechanism the humics interfere with recovery of these compounds, but several are possible. An association between humics and the organics is one possibility. Carter and Suffet (1983) found that a high degree of organic-humic complexing is correlated with a high octanol-water partition coefficient (K_{OW}). These compounds all have high K_{OW} values, as evidenced by their high methylene chloride-water partition coefficients. Also, the low pH (3) used in this experiment would favor stability of organic-humic complexes since at low pH humics are less charged and therefore less polar than at high pH. The association of humics and organics may be strong enough to decrease the extraction efficiency of some compounds when humics are present. Humics may catalyze degradation of the compounds, either by providing a more suitable environment for bacteria or by directly catalyzing chemical breakdown, e.g. hydrolysis. A third possibility is that the association of humic+organic may bind to the drum surface, thus removing a portion of the humics from the extraction system, resulting in lower aparent extraction recoveries. It must be stated that initial evidence by Carlberg and Martinsen (1982) indicates that the humic-organic equilibrium is very slow for some compounds, requiring from a few days to several months, depending upon the compound. Carter and Suffet (1983) found that humic-organic associations for some compounds reach equilibrium in less than one day. Experimental factors such as mixing could be important in the kinetics of organic-humic associations.

The partitioning of Fluka humic material was studied at pH 3 and pH 7 to ascertain the amount of potential interference with subsequent GC analysis (Gibs and Suffet, 1985). E and p-values were determined by quantifying the humic concentration in pH 3 and 7 salt solution by UV analysis at 254 nm of the humic material before and after methylene chloride extraction. The method

followed the procedure of Suffet and Faust (1972). Values obtained were:

pH 3		pH 7	
********		********	
p-Value	10:1 E-Value	p-Value	10:1 E-Value
0.48	0.07	0.19	0.02

Less humic material is extracted at the high pH as the charge of the humic material increases when the acid groups are dissociated. Humics would adversely effect the GC column and interfere with GC analysis, so base extraction would be needed for samples containing high humics at pH 3 or pH 7 to protect the GC column (Gibs and Suffet 1985).

pH 7 vs. pH 3 Extraction

Since all pH 7 extractions were completed without humics present, only non-humic pH 3 data will be used to compare pH 3 and pH 7 recoveries. Rigorous statistical comparisons between pH 3 and pH 7 extraction recoveries were not possible due to the small number of pH 7 replicate values. However, some semi-quantitative observations can be made. As previously reported (Baker, et al. 1985) partition coefficients for 12 of the 14 compounds are virtually identical for pH 3 and 7. The partition coefficients are the primary controlling factor in extraction recovery for both batch LLE and CLLE. The only operational differences between CLLE operation at pH 3 versus 7 were those caused by rapid biological growth at pH 7. This growth may decrease recoveries at pH 7 by metabolic breakdown of compounds and/or by interfering with extraction and separation. Recoveries of four compounds are compared at pH 3 and 7 below:

COMPOUND	pH 3 BATCH	pH 7 BATCH	pH 3 CLLE	pH 7 CLLE
Acetophenone	93%	72%	101%	81%
Isophorone	81%	78%	82%	87%
Biphenyl	64%	60%	70%	47%
Caffeine	37%	40%	33%	42%

Recovery trends at pH 7 appear to follow those measured at pH 3. Acetophenone shows the most significant pH effect. More pH 7 data would be needed for a statistical comparison. A qualitative review of the data indicates that pH 3 and pH 7 extraction recoveries are not substantially different for batch LLE of for CLLE. The current standard CLLE procedure specifies pH 3 to control biological growth.

CONCLUSION

A Teflon helix CLLE apparatus was adapted for use in water sampling for biological testing. The CLLE was shown to be reliable enough to be used for long-term composite sampling.

Statistical evaluations showed close agreement between batch LLE and CLLE recoveries for the compounds tested, both with and without humics present. Some decrease in recovery was noted after humics were added to the aqueous sample for three of the nine compounds where this comparison was possible.

pH 3 was selected as the standard operating pH for the CLLE due to biological growth at pH 7 and salt precipitation at higher pH ranges. pH 3 extraction recoveries appear to be equivalent to pH 7 values for compounds which do not dissociate in that pH range.

The current version of CLLE apparatus is field-ready and suitable for sampling for biological testing, and sampling currently underway will give an indication of the future role of this sampler.

Figure 1

Isolation Scheme to Collect Extracts for Animal Studies

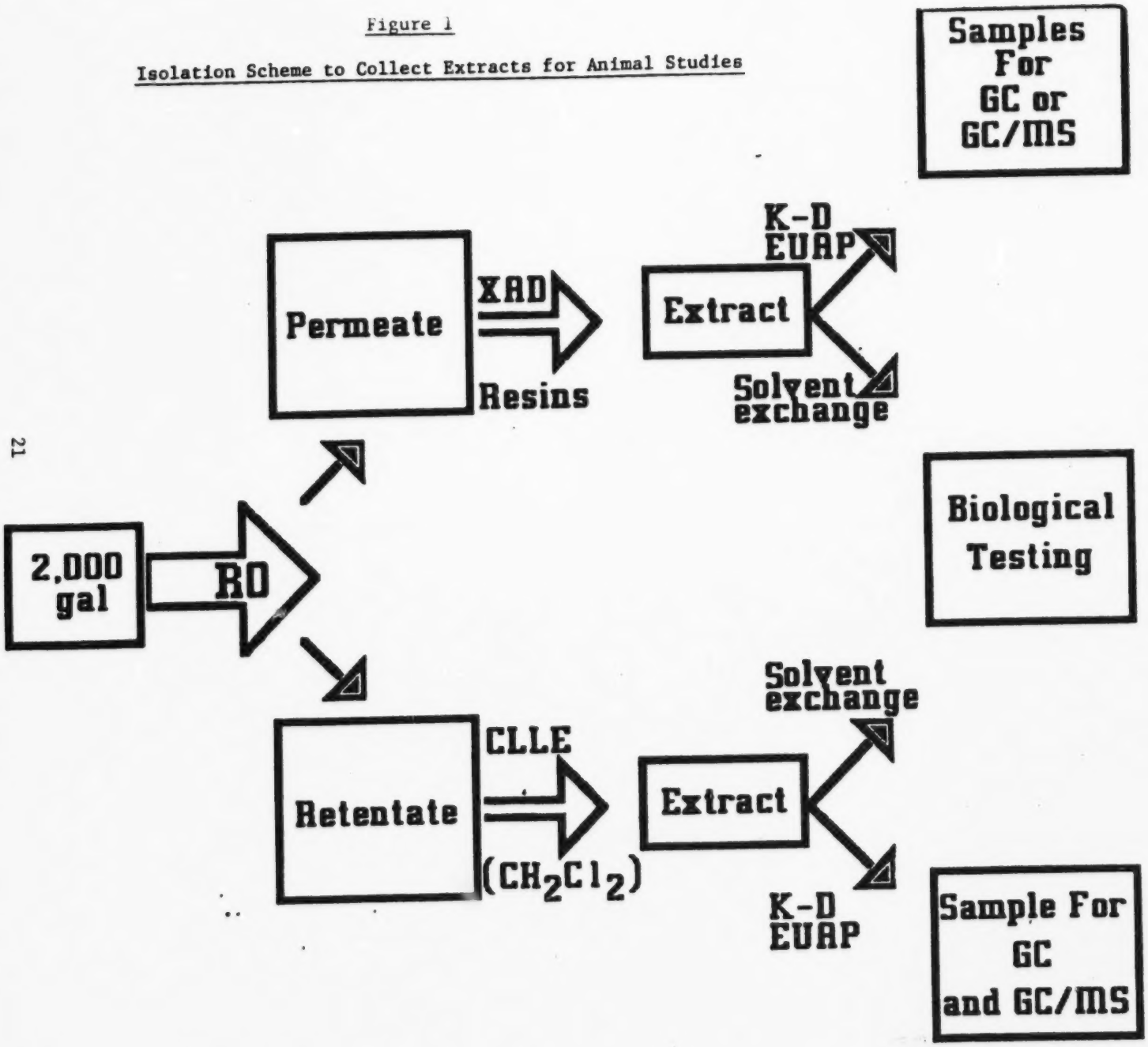
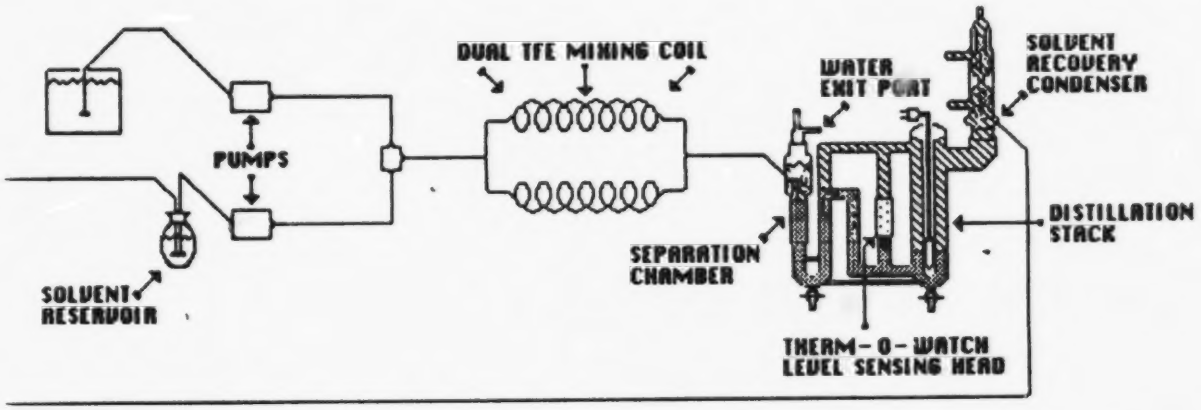


Figure 2

Schematic Diagram of the Continuous Liquid-Liquid Extractor



x Second Large Scale CLLE Extraction Experiment of 100 Liters at pH = 3

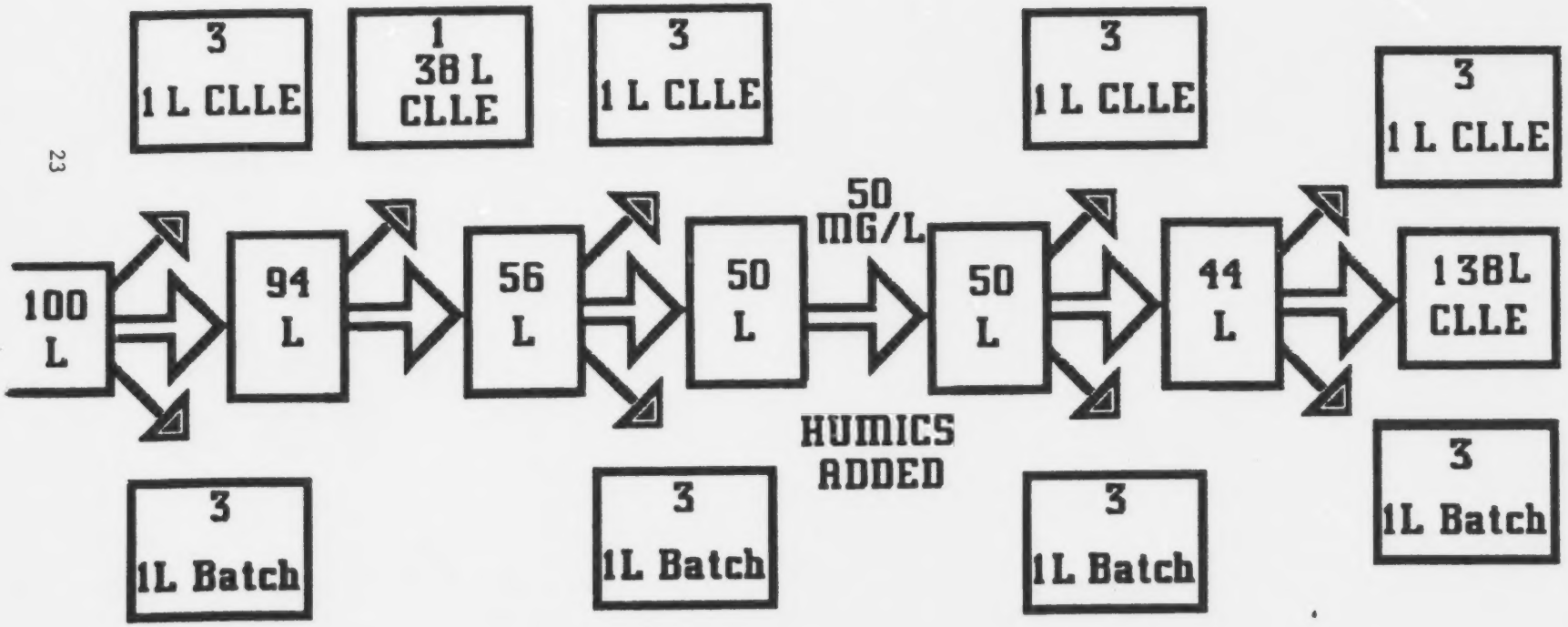


Figure 4

Third Large Scale CLLE Extraction Experiment of 100 Liters at pH = 3

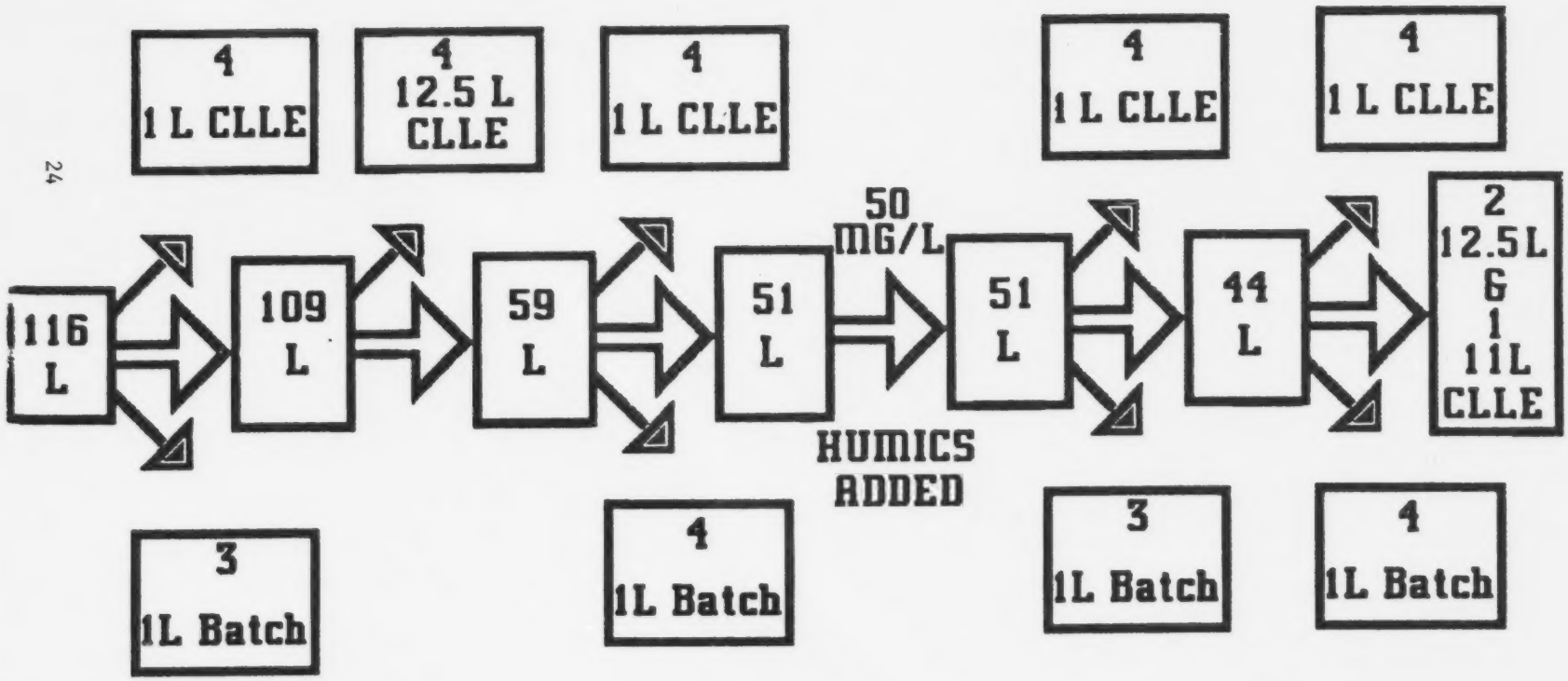
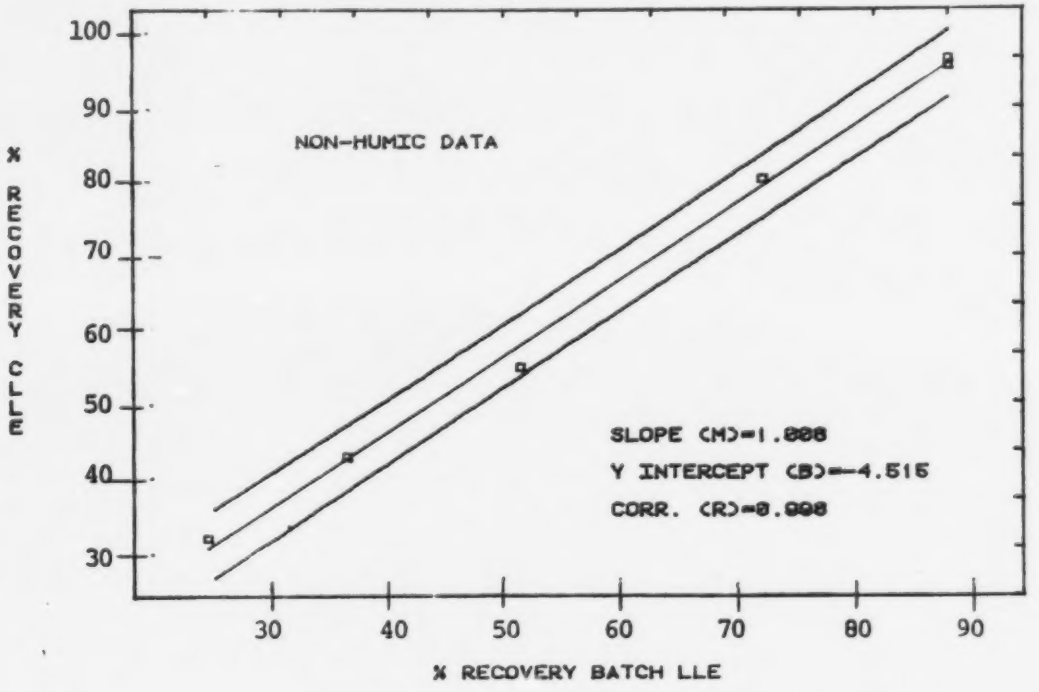


Figure 5. Linear Regression Analysis of 1 L Batch vs. 1 L Continuous Liquid- Liquid Extractions- Experiment #2



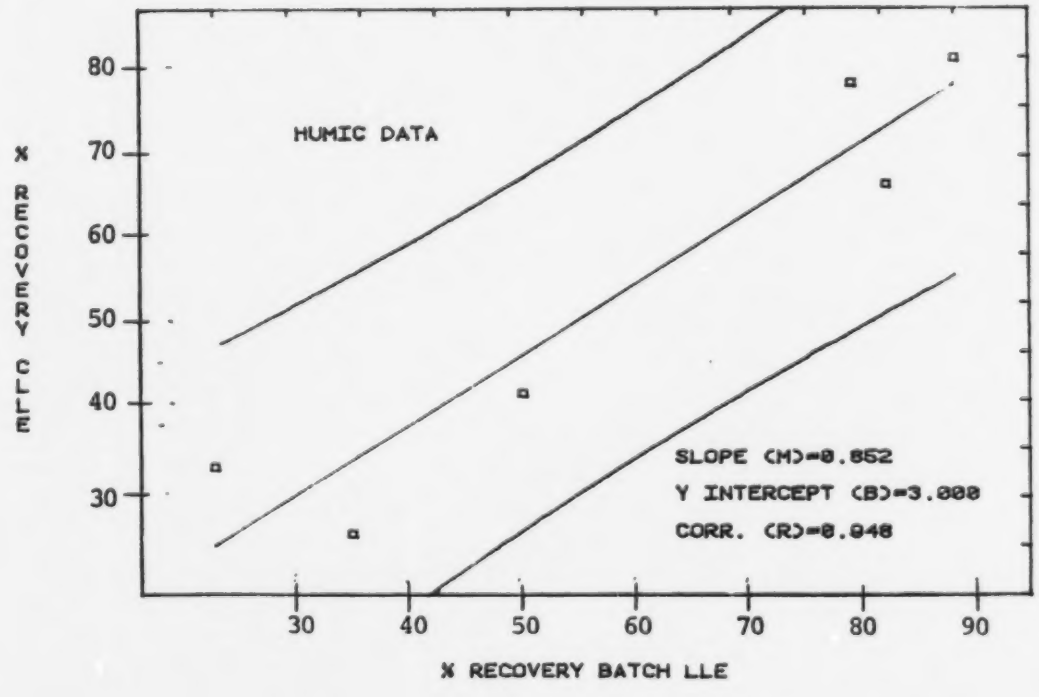
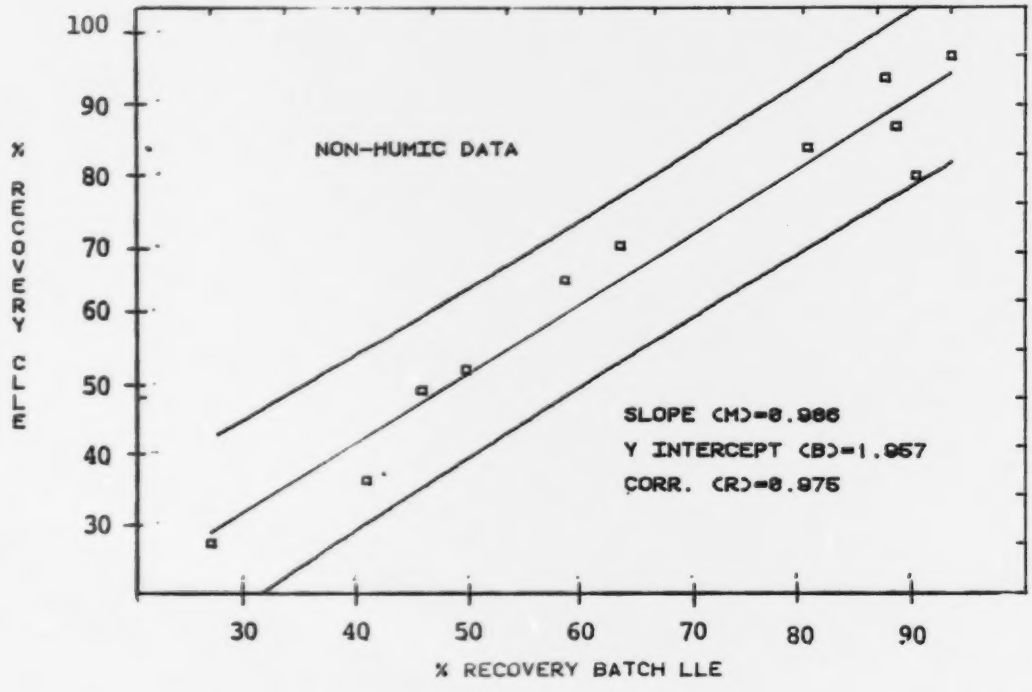


Figure 6. Linear Regression Analysis of 1 L Batch vs. 1 L Continuous Liquid- Liquid Extractions- Experiment #3



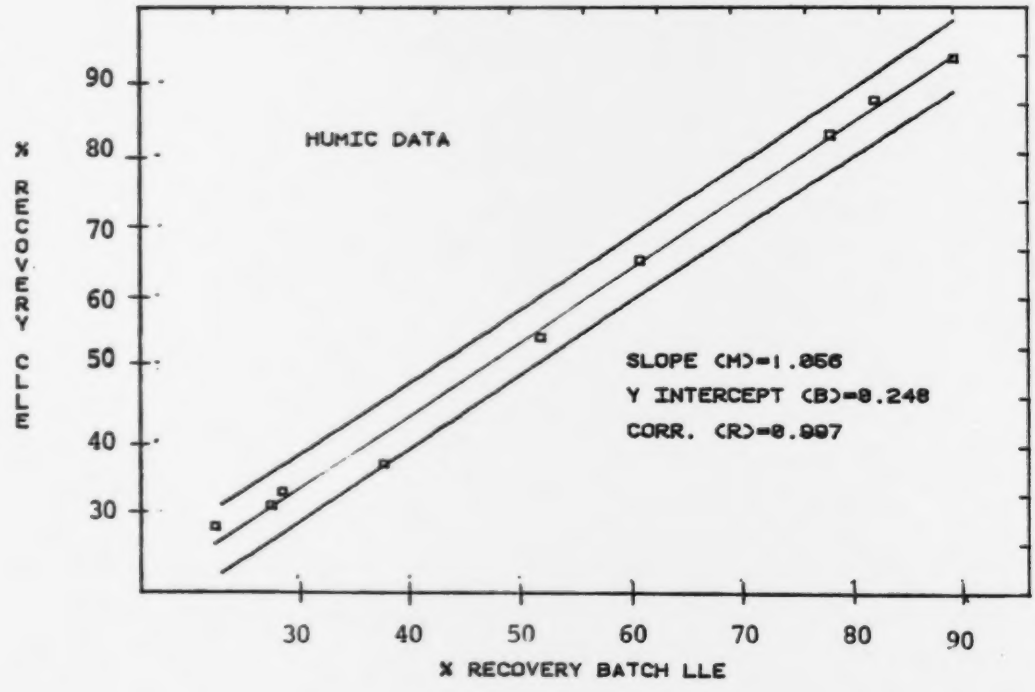
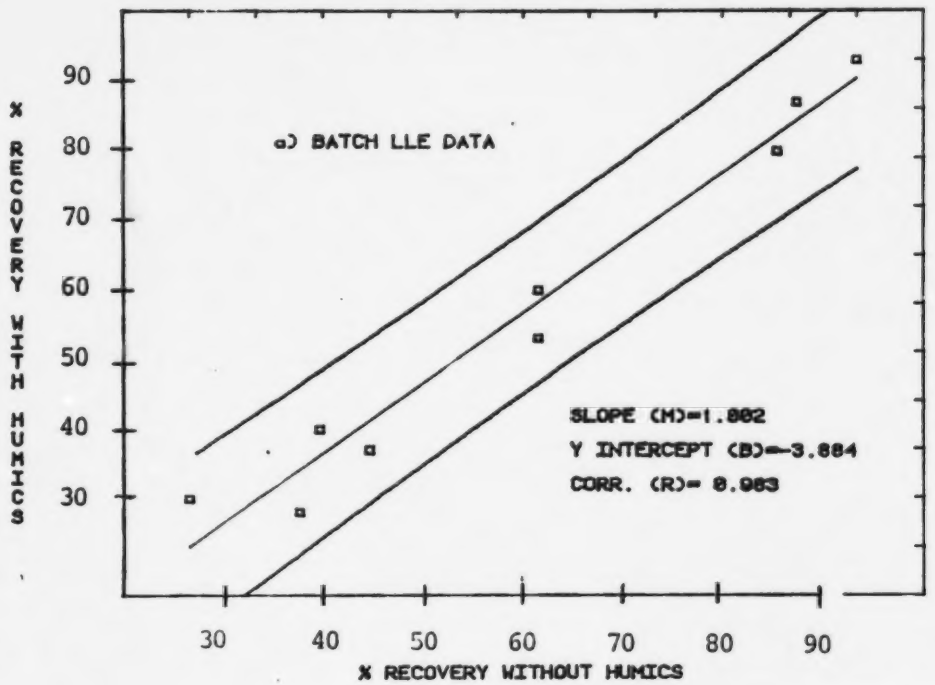
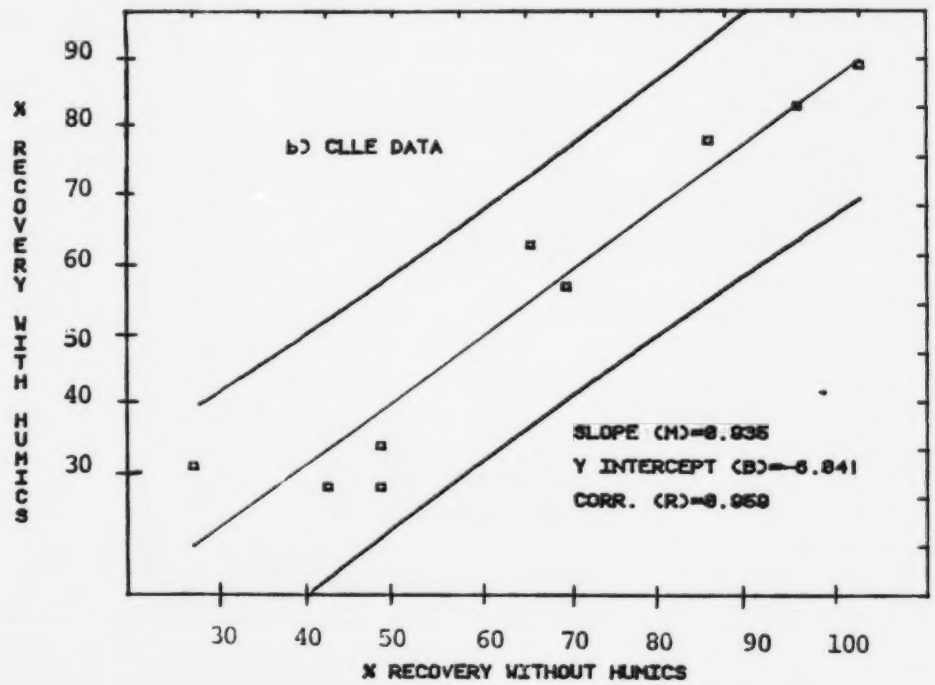


Figure 7. Linear Regressions of Humic vs. Non-Humic Recovery Data for 1 L Batch vs 1 L Continuous Liquid-Liquid Extractions





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